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Review

Fecal microbiota transplantation in human metabolic diseases: From a murky past to a bright future?

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SUMMARY

Fecal microbiota transplantation (FMT) is gaining considerable traction as a therapeutic approach to influence the course of a plethora of chronic conditions, ranging from metabolic syndrome and malignancies to auto-immune and neurological diseases, and helped to establish the contribution of the gut microbiome to these conditions. Although FMT procedures have yielded important mechanistic insights, their use in clinical practice may be limited due to practical objections in the setting of metabolic diseases. While its applicability is established to treat recurrent *Clostridiodes difficile*, FMT is emerging in ulcerative colitis and various other diseases. A particularly new insight is that FMTs may not only alter insulin sensitivity but may also alter the course of type 1 diabetes by attenuating underlying auto-immunity. In this review, we will outline the major principles and pitfalls of FMT and where optimization of study design and the procedure itself will further advance the field of cardiometabolic medicine.

INTRODUCTION

Most medical research has focused on alterations in human cells as the major cause of chronic diseases, while microbes were mainly studied in the context of infectious diseases and largely neglected for their role in human health. In hindsight, this may be somewhat surprising, as Antonie van Leeuwenhoek already discovered the presence of micro-organisms in his own oral cavity several centuries ago. Moreover, current analyses even estimate that the microbial cells carried on the average human body are at least equal in number compared to human cells (Sender et al., 2016). In its entirety, our commensal microbial cells are thought to weigh up to approximately one kilogram, with the vast majority residing in the gut. These commensal microbial lifeforms, encompassing bacteria, archaea, viruses, and fungi and in some cases also including protists (collectively termed microbiota), are together with their theater of activity referred to as the microbiome (Berg et al., 2020). One of the most important assets of the fecal microbiota transplantation (FMT) is the fact that some form of causality can be established in microbiome research, although it should be noted that fecal transplants contain more than the microbiota or microbiome, and even include human colonocytes (Figure 1) (Bojanova and Bordenstein, 2016; de Vos, 2013).

The emerging appreciation for the potential importance of the gut microbiome for human health and disease stems from a few key innovations and clinical observations. First, the broad use of antibiotics was found to cause profound perturbances of the gut microbiome, a state referred by some as dysbiosis, but as the extent of deviations may vary considerably, we prefer to refrain from using this term. In some cases, this can lead to the dreaded recurrent pseudomembranous colitis, caused by overgrowth of *Clostridiodes difficile* (*C. difficile*). Following the logical reasoning that restoration of the gut microbiome by transplantation of fecal microbiota from an otherwise healthy donor would hamper further outgrowth of *C. difficile*, an FMT has been developed and formally tested in a randomized clinical trial that was stopped early due to striking superiority of FMT above other treatments, including vancomycin treatment, in reducing recurrent episodes of diarrhea (van Nood et al., 2013). However, fecal transplantation is actually not a new procedure. Perhaps more born out of desperation than mechanistic insight, as far back as 3,000 years ago cow feces were recommended to ail gastrointestinal complaints in India. Furthermore, in China around 400 BC patients were treated with "yellow soup" (a mix of fresh feces and water) for food poisoning and diarrhea (Zhang et al., 2012). In the Second World War German soldiers in Northern Africa were given camel stool as a treatment against dysentery. Currently, FMT is used in clinical practice for recurring *C. difficile* infection, after several clinical trials have established FMT as a viable treatment for this condition (Kao et al., 2017; van Nood et al., 2013).

Furthermore, with the dawn of ever faster, cheaper, and more detailed high-throughput sequencing techniques and advances in bio-informatics and machine learning, alterations in the gut microbiome have been increasingly linked to non-communicable diseases, such as type 1 and type 2 diabetes (T1D and T2D), various cancers, and auto-immune diseases (Hartstra et al., 2015). A logical next step to address the causal contribution of alterations in the gut microbiome to these conditions has been to perform FMT in these diseases. However, these studies have yielded mixed results. While there is general consensus that

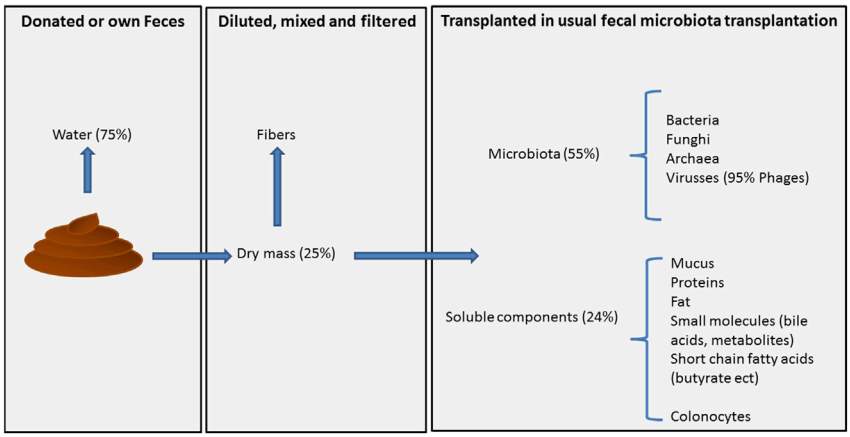

Figure 1. Components that are transferred during fecal transplantation

careful donor selection and screening for infectious diseases is mandated for obvious reasons (Cammarota et al., 2019), the interpretation and comparison between these studies has been hampered by a large heterogeneity in the methods by which metabolic health and diet of the FMT donor is assessed and via which route the FMT procedure is performed. In this review, we will critically appraise the efficacy and effect size of treatment of FMT in human disease, discuss its major pitfalls, and provide a roadmap of how the FMT procedure may be further standardized and optimized to maximize its use in both clinical and research settings.

WHAT CONSTITUTES A FECAL MICROBIOTA TRANSPLANTATION?

Since we do not live in a sterile word, in its basic form FMT is the oral consumption of gut microbiota from either humans or other species. Of note, during our evolution we have been exposed to microbes in a variety of ways, including foods produced by fermentation (Rook, 2010). Moreover, our mouth is regarded as an important reservoir for gut microbiota and vice versa (Schmidt et al., 2019). It is becoming clear that this natural process has implications for human health. Notably, a recent study revealed that children growing up on a farm are at reduced risk of developing asthma, and this phenomenon is at least partly explained by an altered gut microbiome (Depner et al., 2020). Furthermore, infants born by Caesarean section are at increased risk of several auto-immune conditions, while their gut microbiome is altered, as the initial transfer of microbes from the vaginal canal to the infant is replaced by skin microbes from the mother and the surgical team (Dominguez-Bello et al., 2010). The gut microbiome of these infants can be reshaped by supplying a small quantity of maternal feces in mother's milk, leading to a microbial composition that more closely resembles that of infants born through vaginal delivery (Korpela et al., 2020).

For its use in a medical setting, FMT has been optimized to be an odorless and tasteless procedure for the recipient, but the way the procedure is performed varies considerably, ranging from oral daily consumption of freeze-dried capsules as well as single/multiple treatments with small intestinal infusion via a naso-duodenal tube, to transfer via esophago-gastroduodenoscopy or colonoscopy, or a colonic retention enema. Often, the pre-existing intestinal microbial load is reduced by either a bowel lavage or consumption of antibiotics or laxatives.

What is actually transferred is also heterogenous (Figure 1), both in scientific studies as well as in clinical practice. Although the field seems to primarily focus on the transfer of microbiota, and therein on bacteria in particular, the other components of the FMT are often somewhat neglected. This includes attention for bacteriophages, the bacterial viruses that may be causal in the effectivity of FMT in treating recurrent *C. difficile* infections (Ott et al., 2017). Interest in use of bacteriophages is developing and model studies have shown the impact of fecal virome transplantations as reviewed recently (Rasmussen et al., 2020). Only few studies have focused on the abiotic fecal compartment. A meticulous analysis of feces from adults consuming a typical British diet found that on average the fecal discharge approximated 100 g daily, with only 25% making up dry mass (Stephen and Cummings, 1980). The dry mass consisted of 55% microorganisms, while the remainder largely encompassed fibers such as cellulose, and soluble components (24%), such as mucus, discharged colonocytes, proteins, fat, small molecules like bile acids that produce cholesterol degradation products including catechols, indoles, and sulfides (giving feces their characteristic odor), and characteristic short-chain fatty acids (SCFAs), such as acetate, propionate, and butyrate. It is important to realize that in most FMT protocols the feces are mixed with saline and filtered to remove large insoluble particles, while few efforts are made to purify or enrich the microbial fractions. Therefore, potential beneficial effects of FMT may be in part due to these compounds.

Based on metagenome sequencing techniques, the microbial fractions have been estimated to consist of mainly prokaryotes (bacteria and archaea in a ratio of 10 to 1 as reported recently; Kim et al., 2020), while fungi are in the minority (Qin et al., 2010). Viruses often have been estimated to outnumber their hosts, and the human gut is no exception, as many prokaryotes contain prophages while a large and diverse phageome has been reported in healthy subjects (Manrique et al., 2016). These sequencing analyses also revealed that feces contain a considerable amount of human DNA, presumably from excreted epithelial and immune cells. The viability of excreted fecal microbiota has been determined with flow cytometry, finding that almost half of the fecal microbes (49%) are dead, and subsequent sorting of microbes revealed that some species were only found in the dead fractions (Ben-Amor et al., 2005). One may presume that viability will only decrease further, even if greatest care is taken, during FMT sample processing, and this has been addressed recently (Papanicolas et al., 2019).

Autologous fecal microbiota transplantation

Although most studies have focused on transplanting fecal microbiota of a healthy donor (allogenic FMT), there are

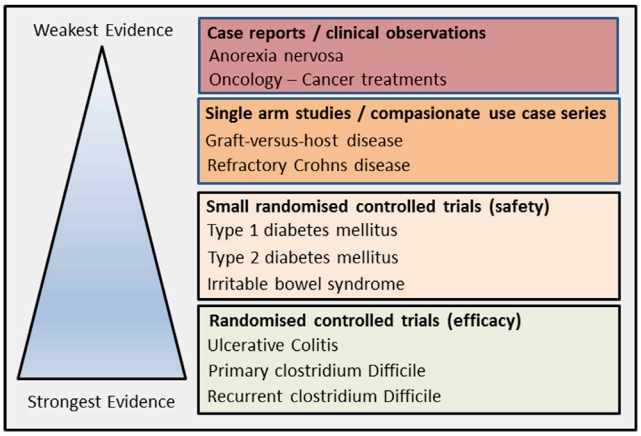

Figure 2. Pyramid representing the strength of clinical evidence that FMT influences clinical outcomes

considerable advantages to using own (autologous) feces whenever possible, such as lower safety concerns for infections, but perhaps also higher effectivity due to better engraftment. For the treatment of recurrent *C. difficile* infections in the setting of hematologic diseases, this approach is best established with banking of frozen feces when the patient's own microbiome is still diverse prior to the conditioning regimen (Taur et al., 2018). However, this approach may also be beneficial in other conditions, where an individual's own fluctuation in microbiome composition contributes to disease severity. A clear example here is formed by the inflammatory bowel diseases, where it is thought that aberrations in the intestinal microbiota contribute to the severity of the disease, while the intestinal inflammation is also thought to influence the composition of the microbiome. Therefore, it has been postulated that biobanking of feces containing a microbiome with beneficial and personalized properties can be used later to abort relapses of disease. An important notion in this framework is that if causative changes in the microbiome occur prior to disease relapse, identification of a clear beneficial signature in the microbiome is needed and it is not sufficient to simply collect feces during episodes of clinical remission (Basson et al., 2020). Following similar reasoning, autologous FMT may also be used to extend the effects of intensive lifestyle interventions that in part improve metabolism through alterations in the gut microbiome. Indeed, a recent study found that autologous FMTs from fecal matter banked during a healthy Mediterranean diet prevented weight regain when the diet was discontinued (Rinott et al., 2021).

The examples above mainly aimed to restore the microbiome of the large intestine. However, when delivered through duodenal tubes or oral capsules, autologous FMT can also be used to profoundly reshape the composition of the small intestine, with a potentially strong impact on the disease course of auto-immune disorders, as training of the immune system largely takes place in the small intestine in response to antigen exposure (Esplugues et al., 2011). We found, for instance, evidence that duodenal autologous FMT delivered via duodenal tubes may preserve beta cell function in newly diagnosed T1D, and this approach seemed more potent than allogenic FMT from a healthy donor (de Groot et al., 2021).

HIERARCHY OF EVIDENCE: FMT IN DISEASE

Can FMT improve hard clinical endpoints?

Although FMTs have been evaluated in many chronic conditions, the strength of the evidence differs considerably by design and study size (Figure 2). These differences may be attributable to variations in transplantation algorithms, study populations selected, and notably small study sizes. Furthermore, FMT studies are often performed in highly treatment-resistant subpopulations, such as refractory Crohn's disease (Cui et al., 2015) and graft versus host disease (Kakihana et al., 2016). These and many other studies are published without a control group, and a comparison is made only to the historical course of these conditions. However, in a small randomized controlled trial (RCT), we showed that compared to autologous FMT, donor FMT prolongs progression-free survival by several months in cachectic patients with advanced gastroesophageal cancer (de Clercq et al., 2021). Although rates of improvement in these small studies are often compelling, these should mainly be considered as safety studies that justify randomized and double-blinded clinical trials. Furthermore, because few randomized clinical trials have evaluated hard clinical endpoints as their main outcome, and calculation of a number needed to treat (NNT), giving a clinically meaningful impression of the efficacy of the treatment is therefore seldomly possible.

Nevertheless, in some conditions, such as recurrent *C. difficile* infection, irritable bowel syndrome, and inflammatory bowel diseases, predefined clinical endpoints have been evaluated. Hence, we calculated NNT in these landmark trials to give an impression of the strength of treatment effect in these conditions, where evidence for treatment effect of FMT is most compelling. Although the point estimates of the NNT may have been impressive at first glance in some of these trials, large confidence intervals are obvious, indicating that there exists large uncertainty how effective FMT really is in reducing semi-hard endpoints (Table 1).

One way to attempt to improve this estimation of treatment effect has been to perform meta-analyses of small studies. These have been performed in the setting of active ulcerative colitis, where despite large heterogeneity in study protocols, a beneficial treatment effect on remission induction was detected by pooling four studies (Costello et al., 2017). For recurrent *C. difficile* infections, network analyses including six studies found donor FMT after vancomycin pretreatment the most effective method to achieve cure (Dembrovszky et al., 2021). However, meta-analyses of small studies should be interpreted with great care, since biases may be systemic across all trials (Packer, 2017). To name a few, inadequate blinding (or in fact open label design), choice of placebo arm, and publication bias may all skew the results toward a type I error. Although these meta-analyses may certainly be useful to more objectively estimate whether FMTs hold any promise in certain disease settings, they cannot replace the urgent need to move toward larger clinical trials, with more stringent randomization and blinding methods, use of a gold-standard primary outcome measurement, longer follow-up times, and importantly a more standardized and carefully designed placebo arm.

Table 1. Randomized trials using FMT illustrating large variation and uncertainty of treatment efficacy

Disease	FMT mode	Control treatment	Follow-up	Endpoint	NNT and 95% CI	ARR and 95% CI	Reference
Primary *C. diff*	anemia, healthy donor (n = 9)	metronidazole (n = 11)	70 days	no recurrent disease	4 (??–??)	32.3 (−7.7 to 72.4)	(Juul et al., 2018)
Recurrent *C. diff*	bowel lavage and duodenal infusion, healthy donor (n = 16)	bowel lavage and oral vancomycin (n = 13)	10 weeks	resolution of diarrhea	2 (1.1–3.5)	58.2 (28.3–88.0)	van Nood et al., 2013
Ulcerative colitis	colonoscopy delivered, healthy donor (n = 38)	colonoscopy delivered, autologous (n = 35)	8 weeks	steroid-free remission	5 (2.5–18.0)	23.0 (5.6–40.5)	(Costello et al., 2019)
Active ulcerative colitis	enema, healthy donor (n = 38)	enema, water (n = 37)	7 weeks	remission	6 (2.8–27.1)	19.6 (3.7–35.5)	(Moayyedi et al., 2015)
Slow-transit constipation	6 FMTs (n = 30)	education, laxatives (n = 30)	12 weeks	3 complete spontaneous bowel movements/week	5 (2.3–44.8)	23.3 (2.2–44.4)	(Tian et al., 2017)
Recurrent hepatic encephalopathy	5 days antibiotic pretreatment, enema, "rational" donor feces (n = 10)	standard care (n = 10)	5 months	further hepatic encephalopathy	2 (1.2–5.3)	50.0 (19.0–81.0)	Bajaj et al., 2017

FMTs IN METABOLIC CONDITIONS

Type 2 diabetes mellitus

A recent RCT showed that autologous FMT after diet-induced weight loss maintains the beneficial metabolic profile in these subjects (Rinott et al., 2021). With the observation that FMT from an obese donor can lead to rapid weight gain, a link between the gut microbiome, overweight, and insulin resistance was made (Alang and Kelly, 2015). The opposite also seems the case, as FMT from lean donors at least temporarily improves insulin resistance in obese metabolic syndrome individuals (Kootte et al., 2017; Vrieze et al., 2012). Although another study did not find this effect of donor FMT on glucose metabolism (Yu et al., 2020), it should also be noted that different (non-stable isotope) clamps as well as metabolic phenotyping including dietary intake of the FMT donor were performed. In fact, recent studies using a single-dose encapsulated FMT showed improvement in lipid metabolism (Ng et al., 2021) and insulin resistance, with the latter showing that this effect was potentiated by adherence to supplementation of low-fermentability fiber supplementation (Mocanu et al., 2021). Therefore, concomitant dietary strategies may influence the effect of the FMT on insulin resistance. This is important as adverse metabolic traits can be transferred (de Groot et al., 2020a). In this regard, it is also likely that the metabolic phenotype of the FMT donor can affect gut-brain axis in human subjects (Hartstra et al., 2020).

Type 1 diabetes mellitus

Based on the fact that SCFAs producing microbiota are reduced in T1D (de Groot et al., 2017), and the observation that in non-obese diabetic (NOD) mice T1D incidence is reduced by administration of the propionate-producing *Akkermansia mucniphila* or a prebiotic diet that greatly increased SCFA production (Hänninen et al., 2018), it is hypothesized that restoration of the microbiota aberrations by using a healthy donor would attenuate auto-immunity and beta cell destruction. We recently reported a clinical trial comparing autologous FMT to allogenic FMT from healthy donors as a modality to slow down decline of beta cell function (de Groot et al., 2021). The donor FMT group indeed seemed to show a slower rate of beta cell decline than shown in several major clinical trials (as measured by mixed-meal simulated C-peptide levels). The main finding was, unexpectedly, that after autologous rather than allogeneic FMT the rate of beta cell decline was preserved for 12 months, after receiving 3 subsequent FMTs. Since the autologous FMT was accompanied by profound changes in mucosal microbiota in the small intestine, we hypothesize that exposure of own microbiota (fecal to oral) may reshape the immunological tone arising from the small intestine. Given the lack of efficacy of immunomodulatory compounds for T1D (Skyler, 2018), we argue these results are of great interest, deserving of replication in a larger trial.

Non-alcoholic fatty liver disease

The gut microbiome has increasingly been linked to non-alcoholic fatty liver disease (NAFLD) (Loomba et al., 2019). Moreover, increased fatty liver disease (NAFLD-NASH [non-alcoholic steatohepatitis]) is seen in the majority of obese metabolic syndrome subjects. Since a plant-based diet is associated with a lower risk of NAFLD (Mazidi and Kengne, 2019), and is linked to alterations in the gut microbiome, we reasoned that FMT from vegan donors into individuals with NAFLD would improve the hepatic inflammation scores in metabolic syndrome subjects. Although the study was stopped early due to slow recruitment, we found a trend toward a lower necro-inflammatory histology score and lower hepatic inflammatory gene expression after the vegan FMT (Witjes et al., 2020), important predictors of progression toward NASH that may culminate in liver cirrhosis. In line, another

small FMT trial performed in individuals with NAFLD showed that healthy donor FMT reduced gut permeability, an important trait of NAFLD linked to many adverse health outcomes, although this small study found no obvious effects on MRI-assessed liver steatosis, underscoring the need to use gold-standard endpoints in these trials as no liver biopsy for histology was done (Craven et al., 2020).

PITFALLS

Mode of delivery

After the unsavory use of "yellow soup" in ancient times, the modern applications of the FMT have turned FMT into a tasteless and odorless procedure. As most of the training of our innate and adaptive immune cells is thought to take place in the small intestine (Esplugues et al., 2011) and to allow for advancing further pharmaceutical developments, we favor the duodenal treatment route using oral capsules and/or freshly prepared fecal material, prepared under strict anaerobic conditions. Using this approach, the highest possible transfer of viable aerobic and anaerobic strains is ensured, and makes sure that both the small and the large intestinal microbiome are reshaped by the transfer. Of course, for specific research questions, one can opt for colonic delivery methods (enema or colonoscopy) if it is deemed undesirable to reshape the small intestinal microbiota like in some non-autoimmune diseases or specific distal intestinal aberrations.

For the large-scale trials that will be necessary to determine whether FMT deserves a spot in the management of other conditions than recurrent *C. difficile* infections, donor FMT provided freeze-dried in capsules or as frozen solution in capsules (either from one donor or pooled) rather than fresh materials may be needed to pull off the logistics of larger scale transplantations. A specific FMT method that is gaining traction in this context is the use of capsules containing freeze-dried feces that can be administered orally, bypassing the need for invasive procedures and complicated logistics of having the donor and the recipient repeatedly visit the hospital on the same day. At least in the setting of *C. difficile*, frozen or freeze-dried formulations seem to be equally effective when either delivered as an enema (Lee et al., 2016) or even as capsules, as reviewed recently (Gulati et al., 2020). However, for conditions in which more subtle perturbations of the microbiome are restored, this approach may be less appropriate, and usually falls under different GMP regulations than the use of fresh fecal material.

Processing of FMT

Protective measures are usually taken to reduce exposure to oxygen as this kills anaerobic bacteria, but these efforts are in general never complete. Composition of the microbiota before or after dilution and filtering under careful anaerobic conditions did not show major differences in our hands (E.G. Zoetendal and W.M.d.V., unpublished data), although the viability of the preparations may have been affected (Papanicolas et al., 2019). Similarly, although prolonged freezing of fecal samples at −80°C seems to largely preserve composition (Carroll et al., 2012), its effect on viability is unclear. However, some reassurance in this regard was provided by a recent study showing in impressive detail that autologous FMT from a transplant stored at −80°C in glycerol quickly and nearly completely restored both the luminal and mucosal microbiome after an antibiotic regimen (Suez et al., 2018). Whether these findings also hold true in the setting of conditions where FMT aims to correct more subtle microbiome deviations remains to be determined.

Placebo

Many of the highly cited FMT studies have used an open label design. However, in FMT studies it is notoriously difficult to design an optimal placebo. At first glance, transplantation of autologous feces may seem ideal for etiological studies. However, when using oral capsules or a naso-duodenal tube, introduction of large-intestine species profoundly changes the composition of the small intestinal microbiome, and since the small intestine is essential for antigen presentation and production of incretin hormones, to name just a few functions, autologous FMT cannot reasonably be considered an inert procedure. Indeed, we found that autologous FMT may be more potent to slow decline of residual beta cell function in T1D than allogeneic FMT (de Groot et al., 2021). The use of brown-colored saline or other forms of liquids may also be problematic, as duodenal infusion of feces was associated with mild and transient cramping in some individuals (van Nood et al., 2013), which may unblind a subset of participants and subsequently the investigators to treatment allocations. These considerations are particularly important when evaluating FMT for self-reported outcomes.

Diversity of the gut microbiome

It is now clear that each individual carries a distinct microbiome, and that on a population level the microbiome shows distinct clustering within ethnic origins, even when these individuals reside in the same geographic region (Deschasaux et al., 2018). Indeed, baseline diversity and gut microbiota composition seem to affect the treatment response of the donor FMT, at least for insulin resistance (Kootte et al., 2017; Yu et al., 2020). As altered gut microbiota composition is related to different dietary compound processing into toxic metabolites, this notion can have important implications. Based on the hypothesized loss of gut microbial diversity over the last 100 years due to our Western lifestyle, a recent position paper compared this intestinal state to a forest ecosystem (Gibbons, 2020) (Figure 3). It is proposed that primary bacterial species (such as complex polysaccharide degraders, including *Desulfovibrio* spp.) are important to increased gut microbiota diversity and thus pave the way for a bloom of secondary species SCFA producers (such as *Bacteroides* or *Eubacterium* spp.) and, ultimately, tertiary species including bacteria that require a recovered ecosystem to thrive (such as *Anaerostipes* spp.). Thus, preselecting FMT donors based on the presence of these specific microbiota strains could be a viable approach to improve clinical outcomes. Therefore, an important tool to better help interpret FMT studies is a detailed tracking of baseline and post-FMT microbial composition (Li et al., 2016). By comparing the microbial composition of the donor and the recipient at baseline and over time, it is possible to track how much of the donor microbiota strains carried over into the recipient. Given the large heterogeneity of microbial species transferred, this information may help clarify when conflicting effects by FMT are found across studies or between subgroups. The most commonly used method for this approach is

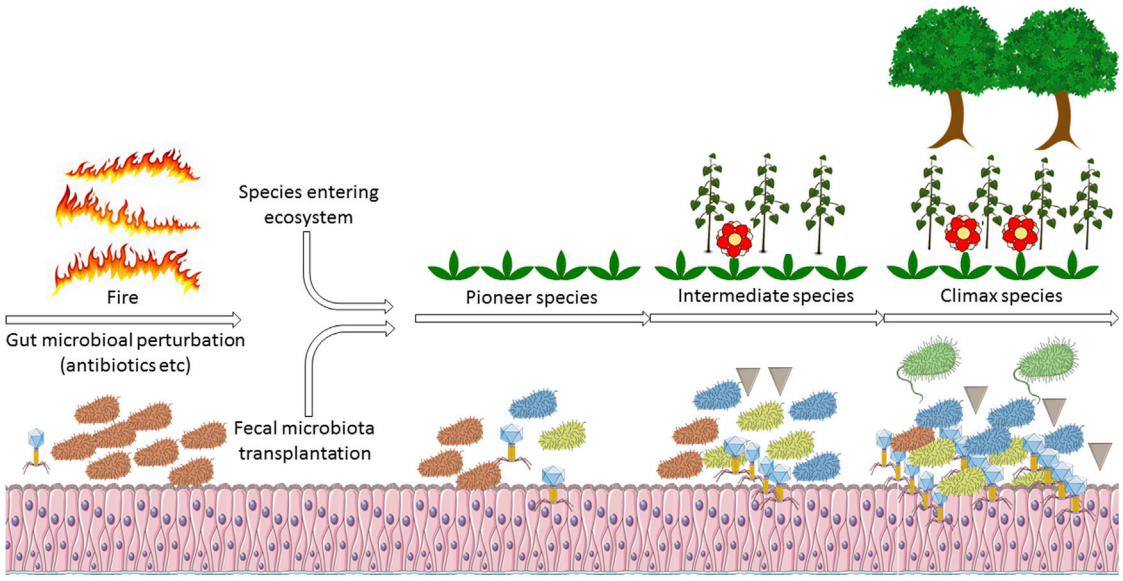

Figure 3. Reconstitution of gut microbiota diversity by FMT-derived key bacterial species

The reconstitution of the gut microbiome after a perturbation (by antibiotics, diet, etc.) is similar to the recovery of a forest ecosystem after a fire (Gibbons 2020). We propose that this recovery can be aided by fecal microbiota transplantation (FMT), similar to species that enter an ecosystem to find an increased chance of growing out with pre-existing climax species eliminated, which eventually may contribute to a new equilibrium and recovered species diversity. In the context of FMT, the newly introduced microbiota may outcompete microbiota that were abundant after the perturbation. This figure was created using figures under the CC0 public domain and Servier Medical Art templates, which are licensed under a Creative Commons Attribution 3.0 Unported License.

deep metagenomic sequencing of fecal samples that allows identification of microbial strains based on specific single-nucleotide variations. Coupling sequencing techniques to bio-informatic analyses such as principal component analyses or t-SNE reveals how long donor-attributable strains and overall similarity remain present, and how much the microbial population may shift back to its original composition.

Donor-acceptor combination

Previous studies have implicated a striking similarity in compatibility between donor and recipient between solid organ transplantation and FMTs. First, the ability to secrete blood group antigens (based on polymorphisms in the fucosyltransferase-2 gene) is associated with decreased gut microbial diversity, and may therefore in turn determine successful engraftment from a non-secreting donor to a blood group secreting recipient (Gampa et al., 2017). This may also hold true for the human leukocyte antigens (HLAs). HLA haplotypes are a major determinant in the risk of auto-immune diseases such as T1D, and infants with HLA haplotypes associated with increased T1D risk indeed developed a distinct microbiome (Russell et al., 2019). It, however, remains to be determined whether FMTs later in life are capable of correcting a "high-risk" microbiome attributable to a specific HLA haplotype. Also, secretion of intestinal immunoglobulins that bind bacteria and bacterial components may contribute to treatment efficacy of donor FMT and thus need to be taken into account (Scheithauer et al., 2021).

Although the FMT procedure is in general a safe and well-tolerated procedure, even in immune-compromised individuals with hematological illnesses (van Lier et al., 2020), the field was recently shaken up by the report of two recipients that died, possibly as a result of a multi-resistant strain of *E. coli* transferred after an FMT (DeFilipp et al., 2019). This report delivered an urgent warning that meticulous screening of the donor for infectious diseases is needed to ensure safety of the procedure (Cammarota et al., 2017), even though this practice is cumbersome for healthy donors and may limit their willingness and/or ability to donate (Tariq et al., 2018). Some special consideration is given to the current COVID-19 pandemic, as the SARS-CoV-2 virus has been found in fecal matter of infected individuals (Gu et al., 2020), raising concerns about whether oral-fecal transmission is possible, and by extension whether FMTs should include screening for COVID-19.

Another concern surrounding a suboptimal match between FMT donor and recipient is that certain traits associated with a perturbated gut microbiome are conferred upon transplantation. Most notably, this seems to be the case for metabolic health of the donor ranging from insulin resistance to morbid obesity, where weight gain has been described after receiving an FMT from an obese donor (Alang and Kelly, 2015). This effect has even been observed in a patient with anorexia nervosa (de Clercq et al., 2019). Furthermore, we found in obese individuals that a favorable response from lean donors was mainly driven by the baseline composition of the fecal microbiome (Kootte et al., 2017; Yu et al., 2020). Therefore, another area where donor-recipient matching may be optimized is the selection of donors that "most optimally" restore the microbiota aberrations of the recipient. This has indeed been attempted for hepatic encephalopathy. These individuals usually have a reduced relative abundance of beneficial SCFA-producing families, such as *Lachnospiraceae* and *Ruminococcaceae*. Hence, in a study where a healthy donor was identified from a donor bank with the greatest abundance from these families, recipients from this donor were much less likely to develop recurrent hepatic encephalopathy

(Bajaj et al., 2017). However, given the open label design of this trial, this study did not answer whether these results are attributable to the FMT procedure regardless of the donor, or even the antibiotic pretreatment that was included in the protocol. Nonetheless, this paper provides exciting support for the possibility that donor-to-recipient matching on fecal microbiota composition may be used to improve disease outcomes. A final option is to use the so-called superdonors, individuals that carry a gut microbiome with especially rich diversity that enhances engraftment after FMT; a recent review addresses examples and their potential (Wilson et al., 2019).

Concomitant medication

To aid in colonization of the transferred microbiota (often referred to as engraftment) of the FMT, the donor infusions are often preceded by a bowel cleansing procedure. Among the most commonly used practices are use of enemas, laxatives, or broad-spectrum antibiotics. Although for ulcerative colitis there exists some evidence that antibiotic pretreatment may improve efficacy of FMT (Keshteli et al., 2017), antibiotic use after administration of the FMT is associated with an increased risk of failure of the procedure (Allegretti et al., 2018).

It has been shown that several commonly used medications are associated with reduced microbial diversity or specific overgrowth of intestinal microbes (Zhernakova et al., 2016), most notably the use of antibiotics and proton-pump inhibitors but also anti-diabetic drugs such as metformin (Forslund et al., 2015; Zhernakova et al., 2016). Furthermore, cardiovascular drugs such as statins, anti-hypertensives, and platelet inhibitors, as well as opiates and anti-depressives, were found to affect the gut microbiota composition. Since such observations are very sensitive to confounding factors such as sex and age, these observations should be interpreted carefully and are ideally followed by randomized studies, as the condition for which these drugs are described may be caused by a perturbed microbiome (Vujkovic-Cvijin et al., 2020). The opposite also holds true, as efficacy of specific oncology medication may be improved by using specific donor FMT (Baruch et al., 2021).

Concomitant lifestyle and diet

A potential factor to consider when transferring microbiota form a donor to a recipient is that their difference in microbial composition may in part be due to lifestyle factors that differ between the donor and the recipient. Therefore, if the lifestyle of the recipient does not change toward the behavior of the donor after the FMT, the effects on microbial composition are likely to dissipate over time. Various intervention studies have indeed compellingly shown that diet shapes the composition of the microbiome, and that changes can occur rapidly with switches in diet, within the course of days or weeks (David et al., 2014; O'Keefe et al., 2015). These changes can be so potent that when an autologous FMT is performed with feces collected during adherence to a specific diet, the beneficial effects of that diet carry over, even when the diet is no longer adhered to (Rinott et al., 2021). Conversely, the individual microbial composition alters the response to dietary changes (Kolodziejczyk et al., 2019; Salonen and de Vos, 2014). Together, these observations argue for the fact that diet may alter the response to FMT by independently shaping the composition of the microbiome. Therefore, the statistical strength of FMT may improve if the diet is standardized during the intervention, as this would remove a large source of variation in the microbiome. This is an overlooked aspect in the field. Exercise and physical fitness per se also seem to influence the composition of the microbiome, although the effects are much weaker than dietary effects (Cronin et al., 2018). The association between alcohol and the microbiome is complicated, as it is thought that alcohol intake influences the composition of the microbiome, but the microbiome may also influence the risk of complications from excessive alcohol use disorder (Bajaj, 2019). The effect of alcohol use in FMT studies is not easily excluded, given how common alcohol use is in many parts of the world. Another lifestyle habit that likely shapes alterations in the gut microbiome is smoking, and given the overt overall negative side effects of smoking, smokers are commonly excluded from donation for FMT. However, smoking is linked to a reduced risk of a few diseases that may be attributable to the effect of smoking on the microbiome. For instance, smoking cessation may lead to microbial alterations that contribute to weight gain (Biedermann et al., 2014). A similar statement has been made in the context between the reduced risk of Parkinson's disease in smokers (Derkinderen et al., 2014). Therefore, counterintuitively, transplantation from smoker's microbiota toward non-smokers may be beneficial in selected settings. Little research has addressed this knowledge gap, likely due to safety concerns that the smoker's microbiome mediates increased risk of heart disease or other tobacco-associated diseases.

Responders versus non-responders

A particularly interesting phenomenon, at least in our hands, is that FMT tends to produce responders and non-responders to the procedure, rather than an overall, or "average," effect (de Groot et al., 2020a, 2021). We hypothesize that the success of the FMT is determined by the extent the donor shifts the microbiome to a more favorable phenotype, and how well engraftment of key species has taken place. This observation was dissected further in a study using non-obese diabetic (NOD) mice, showing that transfer of microbiota from a low T1D incidence colony into mice from a high incidence colony did not alter the underlying diabetes prevalence. Critically, during the FMT not all bacterial strains that differed between the colony were transferred, which may explain why the FMT procedure was not successful in reducing the T1D prevalence. Indeed, when *Akkermansia muciniphila* was transferred, one of the major strains that was not transplanted by the FMT, this single strain reduced the incidence of T1D (Hänninen et al., 2018). A valuable lesson may be learned from this mouse study, in that a single-strain transfer may be more impactful than a full FMT if specific cornerstone species are lost upon transplantation. Therefore, FMTs enriched by certain strains may hold great promise to improve FMT outcomes.

Another exciting approach to improve the response rate after FMT is the selection of patients with a previously favorable disease outcome as donors into individuals with a poor prognosis, yielding a larger clinical benefit than using healthy donors. This was recently demonstrated in a striking study that showed that resistance to check-point blockade therapy could be broken by performing an FMT from patients with a clinical response to check-point blockade in the setting of metastasized melanoma

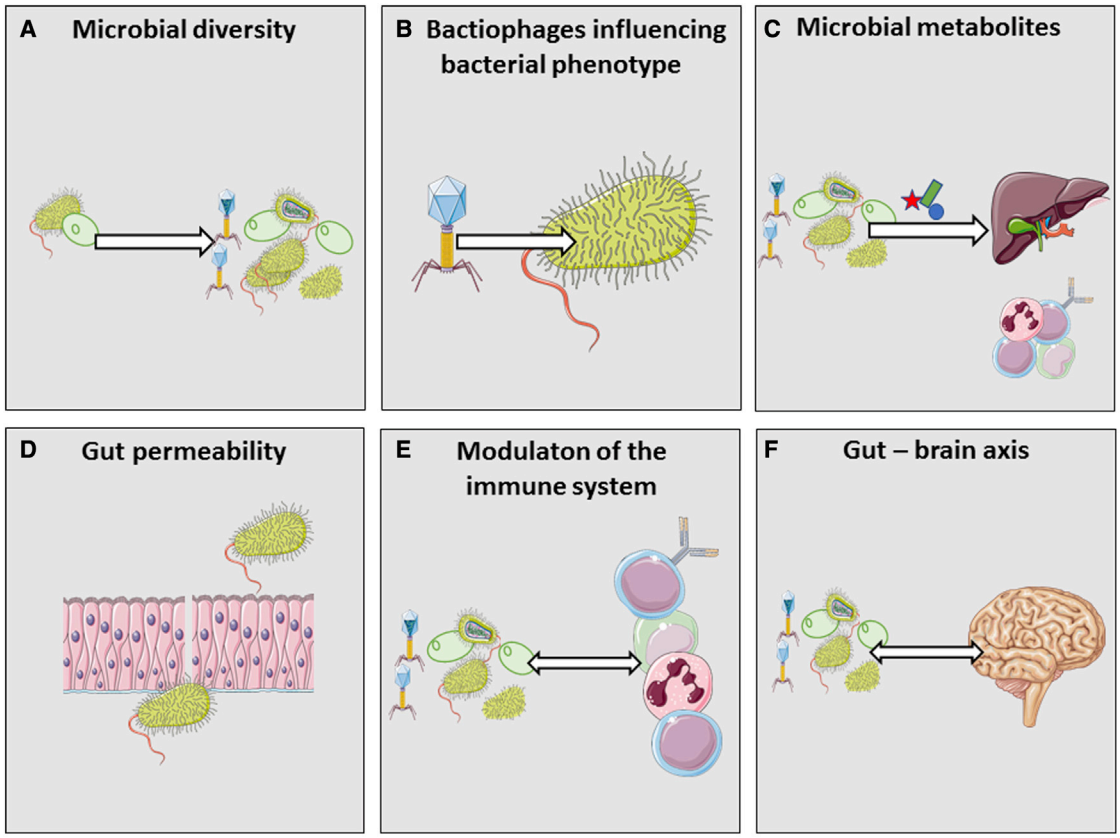

Figure 4. Key mechanisms contributing to modification of microbial and human physiology by FMT
(A) FMT increases microbial diversity.
(B) Bacteriophages transferred by FMT influence gut bacteria by altering their gene transcription and survival.
(C) FMTs alter the overall composition of the microbial metabolites that act on the host (the liver and immune cells pictured are among just a few examples).
(D) FMTs may increase production of specific microbial metabolites, such as short-chain fatty acids (SCFAs), that act as nutrients of the intestine, altering its permeability.
(E) Through altering the production of metabolites, the pool of antigens presented to the immune system, or through mechanisms unknown, FMTs alter the immunological tone of the host.
(F) FMTs alter the microbiome, which in turn acts on the gut-brain axis, potentially leading to alterations in mood and behavior. This figure was created using Servier Medical Art templates, which are licensed under a Creative Commons Attribution 3.0 Unported License.

into anti-programmed cell death protein 1 (PD1) refractory patients (Davar et al., 2021). Indeed, as hypothesized, the responders exhibited increased abundance of taxa that are associated with response to anti-PD-1-based therapy, increased CD8+ T cell activation, and fewer interleukin-8-expressing myeloid cells. This study therefore also provides compelling evidence for the power of FMT to alter the immunological tone of the recipient.

POTENTIAL MECHANISMS OF ACTION

As described above, many FMT studies have been performed in new indications and at most provide evidence for causality of the fecal microbiome while having insufficient power or analysis depth to provide insight into mode of action. Most progress has been made with FMT of recurrent *C. difficile* infections as this treatment has shown that "bugs are better than drugs" (van Nood et al., 2013). It should be noted, however, that this disease is rather unusual since, due to the infection and the recurrent use of antibiotics, the patient's colonic microbiota has a very low diversity with limited microbial interaction networks (Fuentes et al., 2014). Detailed analysis has shown that FMT with a healthy diverse microbiome immediately increased the diversity to normal levels and increased the microbial networks as well as core microbiota (Fuentes et al., 2014; Jalanka et al., 2016) (Figure 4A). A recent study based on deep analysis and mouse studies provided support for the hypothesis that specifically bile salt hydrolase-producing bacteria contribute to the success of the FMT in recurrent *C. difficile* infections, as this enzyme can degrade taurocholic acid, a potent *C. difficile* germinant (Mullish et al., 2019). Of interest, some reports indicate that subsequent disturbances of FMT-treated patients, such as those evoked by the use of antibiotics, may compromise the microbiota and result in a relapse with non-*C. difficile*-dependent colitis that subsequently needed to be corrected by a new FMT (Barberio et al., 2020; Satokari et al., 2014). This situation is reminiscent of a non-resilient microbiome in a metastable equilibrium that can return to an alternative stable state associated with inflammation. This supports earlier postulated models, the most recent of which suggests that the alternative stable states are

associated with increased oxygen concentrations in the colon (Rigottier-Gois, 2013), driving overgrowth of respiring Enterobacteriacea at the expense of sensitive butyrate producers (de Vos and Nieuwdorp, 2013; Zoetendal and de Vos, 2014). Further experimental evidence for this resilience model has come from the discovery of tipping points in the human microbiome and the consensus in the existence of enterotypes (Costea et al., 2018; Lahti et al., 2014).

The largest source of genomic material in fecal matter is formed by the prokaryotic viruses (bacteriophages), as has been recently established (Camarillo-Guerrero et al., 2021). This is phageome is perhaps also the most overlooked in terms of potential contribution to favorable effects of FMT (Figure 4B). This may be undeservingly so, since in the setting of recurrent *C. difficile* infections an FMT depleted of microbes still achieved remission of diarrhea in a small number of patients, suggesting that phages may play an important role in maintaining the health of the host through modulation of the composition and phenotype of gut microbes (Ott et al., 2017). Bacteriophages play a large role in the gene expression of their host bacteria, and even determine their survival. Therefore, beneficial FMT effects may also be mediated through an alteration of the recipient's own bacteria by the donor's phages. This notion becomes even more conceivable when considering that most of the bacteria transferred during the FMT procedure may be dead, and we indeed found in the setting of the metabolic syndrome that responders to an allogenic FMT from a healthy donor maintained a fecal phageome that was more similar to the donor than the non-responders (Manrique et al., 2021). A recent study addressed the development of the microbiome and virome after FMT in patients with recurrent *C. difficile* infections and showed the concomitant depletion of Proteobacteria and their bacteriophages by the FMT (Fujimoto et al., 2021). Currently, phage-specific transplantations are achieved by performing sterile filtration (Rasmussen et al., 2020). A potential advantage of this approach is that the risk of bacterial infections is reduced, although viral infections are usually not prevented by this approach. Furthermore, there is a need for large-scale human trials to show the potential of bacteriophage-based transplantations in relevant indications.

Given the heterogeneous composition of fecal transplants (Figure 1), the beneficial effects of FMT are likely achieved through a pleiotropic mechanism that may even differ for each of the conditions for which FMT may prove beneficial. Nonetheless, a likely mechanism to be of major importance is an altered production of microbe-associated plasma metabolites (Figure 4C). These may already be present in the transplant, or be subsequently produced by the newly colonizing microbes.

The microbiome produces a wealth of small molecules, most of whose function and effect on the host remain to be determined. To discuss each individually would be beyond the scope of the current review, but perhaps the best-known microbial metabolite is the SCFA butyrate, which is produced by many fiber-fermenting strains, and is thought to reduce gut leakage (Figure 4D), to act as a nutrient for the colonocytes, and to have epigenetic effects. Furthermore, this compound displays anti-inflammatory properties and reduces the incidence of T1D in NOD mice (Jacob et al., 2020). Therefore, FMTs may alter the activity of the immune system against self-antigens (Figure 4E). In addition, we found that butyrate-producing bacterial strains are decreased in individuals with T1D (de Groot et al., 2017). However, when we supplemented butyrate in high concentrations to individuals with T1D, this did not lead to any obvious changes in immune cell assays (de Groot et al., 2020b), and when we performed FMT studies in T1D, butyrate did not show up in metabolomic analyses as a major mediator of beta cell preservation or immune cell activity (de Groot et al., 2021). One of the limitations is that butyrate and other metabolites may be taken up quickly and the levels in fecal water or serum do not reflect the actual concentrations in the portal vein. Therefore, human studies may greatly underestimate biological effects of microbial metabolites when using non-invasive biomarkers. This phenomenon may in part explain certain discrepancies between rodent and human studies. Moreover, we acknowledge that these intervention studies may be underpowered to fully exclude potential benefits of butyrate in the setting of T1D. Hence, altogether these studies illustrate the point that there is a long and winding road from identification of promising microbial leads in observational studies toward preclinical studies and human intervention studies directed at improving clinical endpoints.

It is also increasingly appreciated that gut microbes may produce a range of metabolites that alter human behaviors, either directly by metabolites that pass the blood-brain barrier, or indirectly through modulation of the autonomous nervous activity of the gut leading to altered satiety and mood. FMTs may therefore target the gut-brain axis (Figure 4F), regulating metabolites associated with mood and satiety, key players in the development of insulin resistance (Hartstra et al., 2020).

Through mechanisms that are incompletely understood, it is also becoming clear that the composition of the microbiome is essential for developmental processes. Germ-free NOD mice seem to be at increased risk of auto-immunity, and this effect can even be mimicked by administering antibiotics early in life (Livanos et al., 2016). Similar effects have been described in mice for the propensity to develop adiposity early in life (Cox et al., 2014). These compelling rodent studies are coupled with striking observational studies that link perturbations of the gut microbiome early in life, caused by early life events such as Caesarean section and antibiotic use, to the development of these conditions (Blaser, 2017). It would be of interest whether FMT could normalize the perturbated gut microbiome as has been shown for Caesarean-section-delivered infants (Korpela et al., 2020). Hence, further research activities into FMT techniques that are highly tolerable may be needed to develop regiments that could be used to correct microbiota deviations in early life.

ROADMAP TOWARD THE FUTURE OF FMT IN RESEARCH AND CLINIC

Despite its limitations, the FMT is currently one of the most important tools to investigate the causal contribution of the microbiome to a range of chronic conditions (Bello et al., 2018). In order to maximize the impact from these studies, effort should be undertaken to further standardize the FMT procedure; i.e., its dose response, mode of delivery, pretreatment, and whether fresh or frozen or alternatively pretreated material is used. Furthermore, better definition of endpoints as well as more

Table 2. Overview of emerging companies developing FMT-based therapies

Company name	Primary target	Treatment
Finch therapeutics	• *C. difficile* • inflammatory bowel disease • autism spectrum disorder • chronic hepatitis B	• allogenic transplantation in capsules • rationally selected microbiota
Maatpharma	• individuals with hematological malignancies	• autologous microbiome restorative treatments
Vedanta	• solid tumors • C. difficile • food allergy • inflammatory bowel diseases	• bacterial consortia in capsule
Seres Therapeutics	• C. difficile • inflammatory bowel disease	• microbiota spore-containing capsule

stringent power calculations are needed to reduce the chance of type I errors and allow for a more precise estimation of the magnitude of treatment effects. We propose that repeated focus meetings will contribute to a consensus in this area as has been initiated recently (Cammarota et al., 2017).

FMT studies also aid in the identification of promising leads using a "multi-level" approach where detailed phenotyping in changes in the gut microbiome, plasma, and/or fecal metabolites and patient phenotype may be linked using sophisticated bio-informatic techniques and machine learning algorithms. This is a powerful approach to detect so-called needles in the haystack (i.e., involved bacterial strains and networks), but a remaining caveat of this technique is that many FMT studies are usually designed as safety studies and are therefore small and underpowered. Furthermore, untargeted analyses techniques may be prone to both false-positive and false-negative results. Therefore, most results derived from these studies should be seen as hypothesis generating and be replicated in independent studies.

All in all, the use of donor FMT has the capability to restore gut microbial functionality, resulting in either disease modification or even reversal of human disease. The question in the coming decade will thus be whether targeted microbiota-based treatments, such as FMT with or without added specific bacterial strains, can help to potentiate existing dietary and pharmaceutical therapeutic strategies to improve human health. As employing this approach is cumbersome due to ethical and GMP productional and financial reasons, a key contributor to innovations that may improve FMT benefits and standardize its application is the rise of companies that seek to provide FMT commercial services (Table 2).

In conclusion, to move beyond its last-resort application in the clinical setting of recurrent *C. difficile*, better standardization of FMT techniques is urgently needed. We argue that updated guidelines and companies that offer GMP-grade services are major players to achieve this goal. In addition, large clinical studies with formal power calculations on hard clinical endpoints are warranted now that safety issues have largely been addressed. In addition, the beckoning revolutions in untargeted molecular analyses and bioinformatics allow for more detailed analyses on the potential mechanisms of the FMT procedure. These detailed analyses may identify promising microbial and metabolic leads that may be enriched as pro-, pre-, and postbiotic treatments to potentiate the effects of FMT, or in fact even replace the FMT procedure to finally move the gut microbiome as a therapeutic target for metabolic diseases into the clinical arena.

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AUTHOR CONTRIBUTIONS

N.M.J.H., W.M.d.V., and M.N. wrote, edited, and approved this manuscript.

DECLARATION OF INTERESTS

M.N. and W.M.d.V. are founders, hold stock in, and are members of the Scientific Advisory Board of Caelus Health, the Netherlands. W.M.d.V. is co-founder of and holds stock in A-Mansia, Belgium. M.N. is on the Scientific Advisory Board of Kaleido Biosciences, USA. N.M.J.H. has received honorarium from Boehringer Ingelheim but has no conflicts of interest to report relevant to this publication.

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